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IS 7128 (1973, Reaffirmed 2005): Specification for Proteose Peptone, Microbiological Grade. UDC 577.156.41 : 663.1



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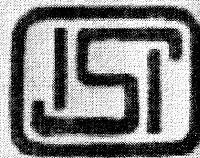
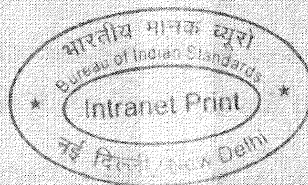
IS: 7128-1973

(Reaffirmed 2005)

Indian Standard

SPECIFICATION FOR PROTEOSE PEPTONE, MICROBIOLOGICAL GRADE

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*Indian Standard*SPECIFICATION FOR PROTEOSE PEPTONE,
MICROBIOLOGICAL GRADE

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(Continued on page 2)

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IS:7128-1973

(Continued from page 1)

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Indian Standard

SPECIFICATION FOR PROTEOSE PEPTONE, MICROBIOLOGICAL GRADE

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 26 November 1973, after the draft finalized by the Food Hygiene, Sampling and Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Unless the ingredients used in media for microbiological work are of uniform quality, the results obtained might be erroneous and might be unreliable. Since the media used in different laboratories often differ greatly in their quality, the results of microbiological work at different laboratories cannot be compared. Therefore, with a view to unifying the practices of different laboratories dealing with microbiology and providing guidance to the indigenous manufacturers regarding the quality, it was decided to bring out a series of Indian Standard specifications for ingredients commonly used in media for microbiological work.

0.3 A specialized peptone, prepared by the enzymatic digestion of selected fresh meat, is particularly adopted for use in media for the production of various bacterial toxins. This is a satisfactory nutrient for the propagation of many pathogenic bacteria.

0.4 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test, shall be rounded off in accordance with IS: 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

1.1 This standard prescribes requirements and methods of test for proteose peptone, microbiological grade.

2. REQUIREMENTS

2.1 Proteose peptone, microbiological grade, shall completely dissolve in water.

2.2 One percent solution in distilled water shall have a pH between 5.0 and 7.0 after autoclaving at 121°C for 30 minutes.

*Rules for rounding off numerical values (*revised*).

IS: 7128-1973

2.3 One percent aqueous solution having pH adjusted to 7.0 and after filtration and sterilization by autoclaving at 121°C for 30 minutes shall be clear.

2.4 It shall be able to support and shall not inhibit the growth of micro-organisms when it is incorporated in suitable medium as the sole growth supporting substance. The general guidelines for testing this characteristic are given in 19 of IS: 6854-1973*. With these general guidelines, micro-organisms like *Staphylococcus aureus* or *Escherichia coli* may be used as the test micro-organism.

2.5 It shall not contain any viable micro-organisms when sterilized at 121°C for 30 minutes and tested according to the method given in IS: 5402-1969†.

2.6 It shall give a positive indole reaction when tested in accordance with the method prescribed in Appendix A.

2.7 It shall give a positive methyl red reaction when tested in accordance with the method prescribed in Appendix B.

2.8 The material shall also conform to the requirements given in Table 1.

3. PACKING, STORAGE AND MARKING

3.1 Packing—The material shall be securely packed in well filled wide-mouth containers with tightly-fitting lids.

3.2 Storage—The material shall be stored in a cool and dry place.

3.3 Marking—Each container shall be marked legibly to give the following information:

- a) Name of the material including the words 'Microbiological Grade',
- b) Name and address of the manufacturer,
- c) Minimum net content, and
- d) Batch or code number.

3.3.1 The container may also be marked with the ISI Certification Mark.

NOTE — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act and the Rules and Regulations made thereunder. The ISI Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well-defined system of inspection, testing and quality control which is devised and supervised by ISI and operated by the producer. ISI marked products are also continuously checked by ISI for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

*Methods of sampling and test for ingredients used in media for microbiological work.

†Method for plate count of bacteria in foodstuffs.

**TABLE 1 REQUIREMENTS FOR PROTEOSE PEPTONE,
MICROBIOLOGICAL GRADE**

(Clause 2.8)

Sl No.	CHARACTERISTIC	REQUIREMENT	METHOD OF TEST, REF TO	
			Appendix of this Standard	Cl No. of IS : 6854-1973*
(1)	(2)	(3)	(4)	(5)
i)	Moisture, percent by mass, <i>Max</i>	5	—	4
ii)	Ash, percent by mass, <i>Max</i>	8	—	6
iii)	Amino acid nitrogen, per- cent by mass, <i>Min</i>	1.5	—	8
iv)	Proteose nitrogen, percent by mass, <i>Min</i>	4		—
v)	Peptone nitrogen, percent by mass, <i>Min</i>	8	D	—
vi)	Total nitrogen, percent by mass, <i>Min</i>	14	—	9
vii)	Phosphorus (as P), percent by mass, <i>Max</i>	0.7	E	—
viii)	Sodium chloride, percent by mass, <i>Max</i>	7.0	—	11
ix)	Iron (as Fe), mg/kg, <i>Max</i>	50	—	13
x)	Copper (as Cu), mg/kg, <i>Max</i>	5	—	15
xi)	Zinc (as Zn), mg/kg, <i>Max</i>	25	—	18
xii)	Fermentable carbohydrates	Nil	—	20

*Methods of sampling and test for ingredients used in media for microbiological work.

4. SAMPLING

4.1 The representative samples of the material shall be drawn according to the method prescribed in 3 of IS: 6854-1973*.

5. TESTS

5.1 Tests shall be carried out by the methods prescribed in 2 and in col 4 and 5 of Table 1.

5.2 **Quality of Reagents** — Unless specified otherwise, pure chemicals and distilled water (*see* IS: 1070-1960†) shall be employed in tests.

NOTE — ' Pure chemicals ' shall mean chemicals that do not contain impurities which affect the results of analysis.

*Methods of sampling and test for ingredients used in media for microbiological work.

†Specification for water, distilled quality (*revised*).

APPENDIX A

(Clause 2.6)

METHOD OF TEST FOR INDOLE REACTION

A-1. REAGENTS

A-1.1 Proteose Peptone Water Medium—Dissolve by gentle heating 10.0 g of proteose peptone under test and 5.0 g of sodium chloride in 1000 ml of distilled water. Adjust pH between 8.0 and 8.4 and boil for 10 minutes. Cool and filter through filter paper and adjust the pH between 7.2 and 7.4. Place required quantities in tubes and sterilize at 120°C for 15 minutes.

A-1.2 Kovac's Reagent—Dissolve 5.0 g of *p*-dimethylamino-benzaldehyde in 75 ml of amyl or isoamyl alcohol by heating at 50 to 55°C on water-bath. Cool and add slowly 25.0 ml of concentrated hydrochloric acid (sp gr 1.18, Min). Keep the reagents either in an amber coloured bottle or in a bottle wrapped with black paper to protect it from light. When not in use, keep at 4°C in a refrigerator. The colour of the reagent should be yellow to light brown.

A-2. PROCEDURE

A-2.1 Inoculate a tube of proteose peptone water medium with a bacterial strain known to produce indole from peptone. A suitable strain is *Escherichia coli*. Inoculate another tube of proteose peptone water medium with a bacterial strain known not to produce indole from peptone. Suitable strains are *Enterobacter cloacae* and *Salmonella typhi*. Incubate the inoculated tubes at 37°C for 48 hours. Add to the growth 0.5 ml of Kovac's reagent, shake well and examine after 1 minute. Red colour in the reagent layer indicates the presence of indole.

APPENDIX B

(Clause 2.7)

METHOD OF TEST FOR METHYL RED REACTION

B-1. REAGENTS

B-1.1 Glucose Phosphate Medium—Steam to dissolve in 1000 ml of distilled water, 5 g of proteose peptone under test and 5.0 g of dipotassium hydrogen phosphate (K_2HPO_4). Cool, filter and adjust to pH 7.5. Add 5.0 g of glucose; mix to dissolve and distribute into tubes. Sterilize at 115°C for 10 minutes.

B-1.2 Methyl Red Reagent—Dissolve 0.04 g of methyl red powder in 40 ml of absolute ethyl alcohol. Dilute with distilled water to a total volume of 100 ml.

B-2. PROCEDURE

B-2.1 Inoculate a tube of the medium with a bacterial strain known to give positive methyl red reaction. A suitable strain is *Escherichia coli*. Inoculate another tube of the medium with a bacterial strain known to give negative methyl red reaction. A suitable strain is *Enterobacter cloacae*. Incubate preferably at 30°C for 5 days or alternatively at 37°C for 2 days. Add to the growth 2 drops of methyl red reagent, shake and examine. Red colour indicates positive reaction and yellow colour indicates negative reaction.

APPENDIX C

[Table 1, Item (iv)]

DETERMINATION OF PROTEOSE NITROGEN**C-1. REAGENTS**

C-1.1 Peptone Solution — 2 percent (*m/m*).

C-1.2 Zinc Sulphate

C-1.3 Sulphuric Acid — 1:1 (*v/v*).

C-2. PROCEDURE

C-2.1 Precipitate peptone by saturating the peptone solution with zinc sulphate. Filter the precipitate and wash several times with saturated zinc sulphate solution to remove nonprotein nitrogen. Dissolve the separated insoluble material in dilute sulphuric acid by heating. Determine the proteose nitrogen content by the method given in 9 of IS: 6854-1973*.

APPENDIX D

[Table 1, Item (v)]

DETERMINATION OF PEPTONE NITROGEN**D-1. REAGENTS**

D-1.1 Peptone Solution — 2 percent (*m/m*).

D-1.2 Tannic Acid — 20 percent and 5 percent (*m/v*).

D-1.3 Sulphuric Acid — 1:1 (*v/v*).

*Methods of sampling and test for ingredients used in media for microbiological work.

D-2. PROCEDURE

D-2.1 Mix equal volumes of peptone and tannic acid solutions in centrifuge tubes and place tubes at 4°C for 30 minutes to complete the precipitation. Do not add excess of tannic acid as this will dissolve the tannic acid-peptone complex. Centrifuge and wash the precipitate twice with cold 5 percent tannic acid solution. Dissolve the washed precipitate in dilute sulphuric acid and determine peptone nitrogen content by the method given in 9 of IS:6854-1973*.

APPENDIX E

[Table 1, Item (vii)]

DETERMINATION OF PHOSPHORUS

E-1. REAGENTS

E-1.1 Potassium Dihydrogen Phosphate—Dry in a desiccator over calcium chloride or silica Gel.

E-1.2 Perchloric Acid—60 percent.

E-1.3 Hydrochloric Acid—3 N.

E-1.4 Ammonium Molybdate Solution—2.5 percent solution in 3 N hydrochloric acid. Keep the solution at 38°C for 24 hours. Store in a brown bottle.

E-1.5 1-Amino-2-Naphthol-4-Sulphonic Acid Solution—Grind fine in a porcelain pestle and mortar, 0.2 g of 1-amino-2-naphthol-4-sulphonic acid, 1.2 g of sodium bisulphite and 1.2 g of sodium sulphite. Store in a brown bottle. (This mixture is suitable, hence a larger quantity can be prepared and stored.) Prepare 1.25 percent solution in a distilled water. Prepare the requisite quantity freshly before use.

E-2. PROCEDURE

E-2.1 Digestion—Transfer about 100 mg of sample, accurately weighed, to a 100-ml Kjeldhal flask. Add 5 ml of perchloric acid and digest for about 2 hours on a digestion rack until colourless using glass beads to prevent bumping. Add 5 ml of distilled water washing down the sides of the flask and boil gently for about 10 minutes. Transfer the solution to a 100-ml volumetric flask, washing the digestion flask several times, with small portions of distilled water. Make up the volume and mix thoroughly. Similarly digest as above approximately 13.7 mg of potassium dihydrogen phosphate, accurately weighed, and make up the volume to 100 ml in a volumetric flask. This is standard phosphate solution containing approximately 31 µg per ml of phosphorus.

*Methods of sampling and test for ingredients used in media for microbiological work.

E-2.2 Colorimetric Estimation—Take 0.5 and 1.0 ml each of sample and standard phosphate solution (**E-2.1**) in suitable test tubes. Make up the volume to 2 ml with distilled water. Add 1 ml of ammonium molybdate solution and 6 ml of distilled water, and shake well. Finally add 1 ml of 1-amino-2-naphthol-4-sulphonic acid solution allowing an interval of 30 seconds or 1 minute before addition of the reagent to the next test tube. Mix well. Take the optical density readings after exactly 10 minutes against the blank containing sample or phosphate standard, in a colorimeter at 660 nm or with a yellow-green filter. In case blank is high in phosphorus content and turns blue, repeat the colorimetric estimation with freshly prepared ammonium molybdate solution.

E-3. CALCULATIONS

E-3.1 Phosphorus (as P) in the sample, percent by mass = $\frac{A \times M_1 \times 10}{B \times V \times M_2}$

where

A = optical density of sample,

M_1 = mass in μg of standard phosphorus which gives B reading,

B = optical density of standard phosphorus solution,

V = volume in ml of digested solution of sample taken for estimation which gives A reading, and

M_2 = mass of sample in mg taken for perchloric acid digestion.

INDIAN STANDARDS
ON
FOOD HYGIENE, SAMPLING AND ANALYSIS

IS:

- 2491-1972 Code for hygienic conditions for food processing units (*first revision*)
- 5059-1969 Code for hygienic conditions for large scale biscuit manufacturing units and bakery units
- 5398-1969 Methods for estimation of thiamine (vitamin B₁) in foodstuffs
- 5399-1969 Methods for estimation of riboflavin (vitamin B₂) in foodstuffs
- 5400-1969 Methods for estimation of nicotinic acid (niacin) foodstuffs
- 5401-1969 Methods for detection and estimation of coliform bacteria in foodstuffs
- 5402-1969 Method for plate count of bacteria in foodstuffs
- 5403-1969 Method for yeast and mould count of foodstuffs
- 5404-1969 Code of practice for handling of food samples for microbiological analysis
- 5835-1970 Methods for estimation of vitamin D in foodstuffs
- 5837-1970 Code for hygienic conditions for soft drinks manufacturing units
- 5838-1970 Methods for estimation of vitamin C in foodstuffs
- 5839-1970 Code for hygienic conditions for manufacture, storage and sale of ice-creams
- 5886-1970 Methods for estimation of carotenes and vitamin A (Retinol) in foodstuffs
- 5887-1970 Methods for detection of bacteria responsible for food poisoning and food-borne diseases
- 6540-1972 Code for hygienic conditions for manufacturing and handling of ice for human consumption
- 6541-1972 Code for hygienic conditions for establishment and maintenance of midday school meal programmes
- 6542-1972 Code for hygienic conditions for fruit and vegetable canning units
- 6850-1973 Agar, microbiological grade
- 6851-1973 Meat extract, microbiological grade
- 6852-1973 Bile salts, microbiological grade
- 6853-1973 Peptone, microbiological grade
- 6854-1973 Methods of sampling and test for ingredients used in media for microbiological work
- 6968-1973 Code for hygienic conditions for PAN (betel) stalls and vendors
- 6969-1973 Code for hygienic conditions for handling and sale of refrigerated drinking water
- 7003-1973 Code for hygienic conditions for sago (SABOODANA) manufacturing units
- 7004-1973 Yeast extract, microbiological grade
- 7005-1973 Code for hygienic conditions for production, processing, transportation and distribution of milk
- 7127-1973 Tryptone, microbiological grade
- 7203-1973 Casein hydrolysate (acid digested), microbiological grade

